



## **Vitamin K**

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### **Vitamin K – forms and function**

There are a number of structurally similar, naturally occurring compounds which have vitamin K activity. The major plant form is phyloquinone (vitamin K<sub>1</sub>; usually abbreviated to K<sub>1</sub>). The menaquinones (vitamins K<sub>2</sub>; usually abbreviated to MK) are predominately of bacterial origin. While K<sub>1</sub> has a 20-carbon phytyl side chain, the MKs have multiple prenyl side chains, their number being indicated by a suffix (i.e. MK-n).

In the typical Western diet, K<sub>1</sub> and MK-n account for 90% and 10% of the vitamin K intake, respectively [1]. Dietary MK-n consists of MK-4, MK-7, MK-8, and MK-9 [2], although MKs with longer side chains up to MK-13 can be found in the human liver [3,4]. Menadione (usually abbreviated to K<sub>3</sub>) is a synthetic vitamin K homolog which, despite toxicity concerns and restricted biological activity, is available in some countries as a pharmaceutical vitamin K preparation [5]. The biological activity of K<sub>3</sub> *in vivo* depends entirely on its conversion to MK-4 [6,7].

At the cellular level, the co-factor role of vitamin K for the conversion of peptide-bound glutamate to gamma-carboxyglutamate (Gla) is well established, as is the associated metabolic cycle whereby the vitamin K 2,3-epoxide metabolite generated during gamma-glutamyl carboxylation is salvaged and re-cycled to active vitamin K [8, 9].

The liver synthesises seven vitamin K-dependent proteins that have a crucial role in blood coagulation (factors II, VII, IX and X, proteins C, S, and Z). Other vitamin K-dependent proteins (Gla proteins), with a widespread tissue distribution, have also been identified; this has led to a re-evaluation of the general physiological function of vitamin K and its role in human health. Putative roles of Gla proteins now extend to a diversity of functions, such as the regulation of bone turnover and calcification [10,11], inhibition of vascular calcification [12], and roles in vascular repair processes [13], cell cycle regulation, cell-cell adhesion and signal transduction [14]. Of particular note is the accumulating body of evidence that has linked sub-optimal vitamin K reserves in bone to an increased risk of osteoporotic fracture [15,16] or to reduced bone mineral density [17].

### **Vitamin K deficiency**

Vitamin K<sub>1</sub> is found chiefly in green leafy vegetables, while MK is present in animal tissues. Like other fat-soluble vitamins, vitamin K is absorbed from the duodenum, where it is dependent on bile and pancreatic secretions for solubilisation. Any condition causing the intestinal malabsorption of fat will therefore lead to a secondary deficiency of fat-soluble vitamins, including vitamin K.

Deficient absorption of vitamin K leads to the depletion of its tissue stores, which is indicated by a decrease in circulating levels of the vitamin long before pathological changes develop. Vitamin K deficiency is more prevalent than generally supposed. Cases are often missed, or detected late, due to the use of inappropriate laboratory markers of vitamin K status - commonly the International Normalised Ratio (INR). The INR, which is based on the prothrombin time, is designed to detect bleeding tendencies and is a very insensitive marker of vitamin K status.

## Vitamin K status assessment

Tissue stores of vitamin K are evaluated by the direct measurement of circulating  $K_1$ . This is supported by the analysis of PIVKA-II (under-carboxylated prothrombin – an abnormal species of Factor II that is only detectable in the circulation of patients with suboptimal vitamin K status). By running these two assays in tandem we are able to monitor the two most important determinants of vitamin K status – availability and utilisation.

The serum concentration of  $K_1$  reflects its storage and transport.  $K_1$  is measured using a modified HPLC method with postcolumn chemical reduction and fluorescence detection [18]. In healthy, normolipaemic adults the non-fasting reference range for  $K_1$  is 0.15–1.55  $\mu\text{g/L}$ .

Serum PIVKA-II is determined using a monoclonal antibody (C4B6) to PIVKA-II in an ELISA [19,20]. The C4B6 MAb used in this assay is conformation-specific such that, in the presence of calcium ions, it binds only to the under-carboxylated species of prothrombin and does not cross-react with fully carboxylated prothrombin. Results are expressed as arbitrary units (AU) per millilitre, where 1 AU is equivalent to 1  $\mu\text{g}$  of multiple PIVKA-II species purified from patients treated with vitamin K antagonists. The upper limit of the reference range for PIVKA-II in adults is 0.2 AU/ml (200 ng/ml).

## Patient preparation

Patients should fast for 12 hours before taking blood for the assessment of vitamin K status and should have refrained from taking nutritional supplements for 24 hours prior to venipuncture.

## Specimen requirements

Serum separator tubes (plain gel tubes - available from Biolab on request). If posted, samples must reach Biolab within 24 hours.

## Methodology

Vitamin  $K_1$  - High pressure liquid chromatography (HPLC).  
PIVKA-II - MAb (C4B6) to PIVKA-II in an ELISA format

**Turn around time:** 10 working days.

**Interpretation:** Results will be supported by a full interpretive comment.

## Reference ranges:

### a) Vitamin $K_1$

Fasting adults: Range: 0.17-0.68  $\mu\text{g/L}$  (0.38 - 1.51 nmol/L)  
Median: 0.37  $\mu\text{g/L}$  (0.82 nmol/L)

Non-fasting adults: Range: 0.15 - 1.55  $\mu\text{g/L}$  (0.33 - 3.44 nmol/L)  
Median: 0.53  $\mu\text{g/L}$  (1.18 nmol/L)

### b) PIVKA-II

In healthy vitamin K-replete subjects the concentration of PIVKA-II in serum is expected to be <0.20 AU/ml.

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